

NEWBORN SCREENING

Summer 2003

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and the Newborn Screening Laboratory*



By Dr. Nicola Longo, MD, Ph.D.
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Phenylketonuria is an autosomal recessive disorder (i.e. parents carry one abnormal gene but do not have the disease) resulting from impaired conversion of phenylalanine to tyrosine. It is one of the most frequent metabolic disorders, with a frequency of 1 in 10,000, meaning that 4-5 children will be born with this condition in Utah per year. This disorder is characterized by an increased concentration of phenylalanine and its by-products in body fluids and may result in severe mental retardation if untreated in infancy. The accumulation of phenylalanine causes competitive inhibition of transport of other amino acids required for protein or neurotransmitter synthesis, reduced synthesis and increased degradation of myelin (the insulation of nerves inside the brain), and inadequate formation of norepinephrine and serotonin (chemicals that help the brain to process and transmit signals to the body). Phenylalanine also blocks the formation of the hair and skin pigment melanin, causing hypopigmentation of hair and skin. Untreated children with classic phenylketonuria are normal at birth, but fail to attain early developmental milestones, their head fails to grow and children develop microcephaly with a progressive impairment of cerebral function. Hyperactivity, seizures, and severe mental retardation are major clinical problems later in life. Electroencephalographic abnormalities; "mousy" odor of skin, hair, and urine (due to phenylacetate accumulation); and a tendency to hypopigmentation and eczema complete the devastating clinical picture. In contrast, affected children who are detected and treated at birth show none of these abnormalities. To prevent mental retardation, diagnosis and initiation of dietary treatment of classic phenylketonuria must occur before the child is 3 weeks of age. For this reason, most newborns in North America and Europe are screened by determinations of blood phenylalanine levels. Abnormal values are confirmed using quantitative analysis of plasma amino acids. Dietary phenylalanine restriction is usually instituted if blood phenylalanine levels are $>250 \mu\text{mol/L}$ (4 mg/dL). Treatment consists in a special diet low in phenylalanine and supplemented with tyrosine, since tyrosine becomes an essential amino acid in phenylalanine hydroxylase deficiency. This is provided with special medical food and by assuming a low-protein diet. With therapy, plasma phenylalanine concentrations should be maintained between 120 and 300 $\mu\text{mol/L}$ (2 and 5 mg/dL). Dietary restriction should be continued and monitored indefinitely.



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A number of women with phenylketonuria who have been treated since infancy have reached adulthood and become pregnant. If maternal phenylalanine levels are not strictly controlled before and during pregnancy, offspring are at increased risk for congenital birth defects (congenital heart disease, cleft lip and palate) and microcephaly. After birth, these children have severe mental and growth retardation. Pregnancy risks can be minimized by continuing lifelong phenylalanine-restricted diets and assuring strict phenylalanine restriction 2 months prior to conception and throughout gestation. Ideally, phenylalanine levels should be maintained below 200 $\mu\text{mol/L}$ throughout pregnancy.

Relaxation of treatment after the first few years of life does not cause mental retardation, but impairs brain functioning. At first, high levels of phenylalanine impair judgment and result in changes in the MRI of the brain. These initial changes are reversible if a phenylalanine-restricted diet is re-instituted. If the brain remains exposed to high phenylalanine levels for long time, irreversible changes occur.

Adult patients with phenylketonuria not identified by newborn screening have severe to profound mental retardation and most live in institutions. They tend to have severe behavioral problems, requiring one-on-one assistance around the clock. Some of these patients have responded very well to diet therapy. Diet therapy has not reversed mental retardation, but has significantly improved the behavior, allowing much less strict supervision, improved quality of life, and a more satisfying relationship between the caregivers and the patient.

In summary, phenylketonuria causes severe mental retardation if not treated early in life. The correct diet can prevent mental retardation and allow a near-normal life of affected patients.



Screening for Phenylketonuria (PKU)

By Jana Coombs, RM(AAM), SV,M(ASCP)
Chief, Metabolic Newborn Screening Laboratory

“Screening” refers to the application of a medical procedure or test to people, who may currently have no symptoms of a particular disease, for the purpose of determining their likelihood of actually having the disease. The goal of screening is to reduce morbidity and/or mortality from a disease by detecting the disease in its earliest stages, when treatment is typically more effective. A screening test must be accurate, quantitative, and reliable. Maximum sensitivity is sought to avoid missing any positive specimens; however, a screening test alone does not diagnose an illness. Patients who have a positive result from a screening test will need further evaluation with additional diagnostic tests or procedures.

Historically, the diagnosis of PKU was first made following an abnormal ferric chloride test on urine, but urine-based methods for the screening of newborns are known to be unreliable. Current screening methods are aimed at detecting

increased levels of phenylalanine in the blood. The most widely used method has been the bacterial inhibition assay described by Guthrie and Susi in 1963. This assay, based on bacterial inhibition, uses media formulated to grow *Bacillus subtilis* in an amount proportional to the concentration of phenylalanine in the patient's blood. The procedure provides only semi-quantitative results, is inhibited by antibiotics, and is considered to be labor intensive.

The Utah Public Health Laboratory Newborn Screening section uses the Neonatal Phenylalanine Test Kit produced by PerkinElmer Life Sciences. This kit is intended for the quantitative determination of phenylalanine in blood specimens, dried on filter paper, as an aid in identifying newborns with elevated levels of phenylalanine in the blood. It is also used to monitor the diets of known PKU positive patients in the state.

The procedure is a modification of the fluorometric assay published by Caman and Robins in 1962. The assay is based on the enhancement of the fluorescence of a phenylalanine-ninhydrin reaction product by the dipeptide, L-leucyl-L-alanine (shown below). A succinate buffer is used to optimize the fluorescence and increase specificity. A copper reagent is used to further enhance the reaction and reduce background. This method measures phenylalanine quantitatively in the presence of other amino acids. It has been semi-automated, and produces fewer false positive results than the bacterial inhibition assay. The analytical sensitivity of the assay is typically 12 $\mu\text{mol/L}$ (0.2 mg/dL).

1. Phenylalanine + ninhydrin \longrightarrow Hydrindantin (purple) + CO_2 + phenylacetaldehyde + NH_3
 2. Phenylacetaldehyde + Leu-Ala \longrightarrow Schiff Base + H_2O
 3. Schiff Base + ninhydrin \longrightarrow *5-(O-Carboxyphenyl)-5-hydroxy-3-phenyl-1-(leucyl-alanine)-2-pyrro
one* + H_2O
- *fluorescent compound*

Properly collected specimens are paramount to a reliable test result. Use of collection papers, other than those provided through the Newborn Screening Program, may affect test results. Inconsistently collected samples, such as spots not uniformly saturated, or over saturated with blood; milking or squeezing the puncture during sample collection; exposing the sample to harsh environmental factors, such as excessive heat after collection; or contaminating the specimen with other bodily fluids, alcohol, water, or substances on ungloved hands may cause inaccurate test results. Improperly collected (unsatisfactory) specimens are rejected for testing. An additional specimen will be requested, and testing will be delayed until an adequate specimen is received.

Sensitivity and specificity are measures of a test's ability to correctly classify a person as having a disease or not having a disease. Sensitivity refers to a test's ability to designate an individual with disease as positive. A highly sensitive test means that there are few false negative results, and thus fewer cases of disease are missed. The specificity of a test is its ability to designate an individual who does not have a disease as negative. A highly specific test means that there are few false positive results.

It is desirable to have a test that is both highly sensitive and highly specific. This frequently is not possible. Typically there is a trade-off. For many clinical tests, there are some people who are clearly normal, some who are clearly abnormal,

and some who fall into the gray area between the two. Choices must be made in establishing the test criteria for positive and negative results.

For phenylalanine screening in the newborn population, our lab puts most of its emphasis on a test that is high in sensitivity at the expense of specificity. In other words, there may be some babies that will have a positive phenylalanine test when they really don't have the disease, but we will catch nearly every baby that really does have the disease. As with any other diagnostic test, data obtained with this procedure should be used as an aid to other medically established procedures, and should be interpreted in conjunction with other clinical data available to the physician. Confirmation of positive newborn screening test results is always necessary.

PKU Follow Up Program

By Angie Livingston, RN, BS
Follow Up Program Nurse

After the specimen has gone through the testing process and determined to be abnormal (level >2.1 mg/dl) the case is given to the follow up program nurse. At this time the program nurse reviews the results and begins the notification and tracking process for the patient.



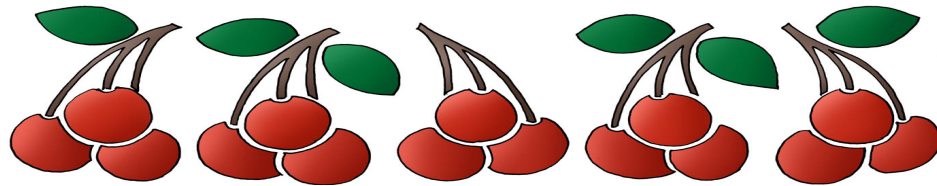
The nurse's first responsibility is to contact the infant's medical home. The medical home is an approach to providing health care services in a high-quality and cost-effective manner. The basis of the medical home is that children receive the care that they need from a pediatrician or physician whom they trust. The follow up nurse gets medical home information from the specimen demographic card. If the card is filled out incompletely or inaccurately reporting of abnormal PKU results can be delayed while this information is tracked down. It is important to make sure every specimen is properly identified and medical home information is accurate.

The nurse's primary responsibility in PKU follow up is to coordinate patient care with the medical home. The metabolic clinic plays an important role in the follow up process for abnormal specimens. Follow up procedures for abnormal results are established on an individual basis.

Most often, confirmatory testing is requested for a second abnormal PKU specimen or if the first abnormal PKU specimen is clinically significant. Confirmatory testing is arranged with the medical home. Confirmatory testing usually consists of Quantitative Amino Acids and Biopterin Determination. Both of these tests are arranged through the newborn screening program and individualized based on patient history. If after confirmatory testing a diagnosis of classical PKU is made the patient is referred to the metabolic clinic for treatment. The follow up program nurse's role ends once the patient is referred and all testing is completed.

PKU screening and follow up is a team effort. Following is a list of problems that delay or produce inaccurate results during the specimen collection process.

- **Missing or incomplete information on demographic card.** It is extremely important for all newborn screening specimens to be properly identified. Demographic information must be complete and accurate to insure timely follow up.
- **Infants on TPN (total parental nutrition)** often have false positive screening results for PKU. Drawing the newborn screening specimen by heel stick, as opposed to an artirial line, can prevent false positive screens.
- **Infants that have received blood transfusions** can also have inaccurate screening results. When feasible the newborn screening specimen should be collected before a transfusion is given. Specimens should be collected 7-10 days after a transfusion. Please check with the newborn screening staff to determine specimen collection timing if infant has been transfused.



Dietary Treatment of Phenylketonuria

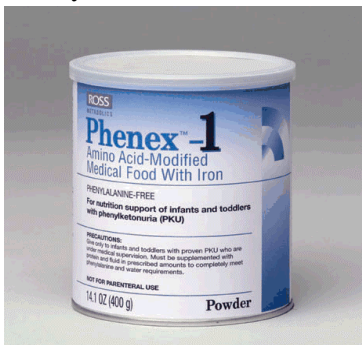
By Sharon Ernst, MPH, RD, CD

Metabolic Nutritionist

U of U, Medical Genetics

Since the late 1950's, a low phenylalanine diet has been used to prevent mental retardation associated with PKU. Treatment should begin as soon after birth as possible in infants with phenylalanine levels over 6 mg/dl (360 umol/L) and should be continued indefinitely.

Phenylalanine is an essential amino acid, therefore, it cannot be totally omitted from the diet. All children need a certain amount of phenylalanine for normal growth and protein synthesis. Usually the unused phenylalanine is converted to tyrosine and is eventually metabolized in the body. Because the child with PKU is deficient in the liver enzyme phenylalanine hydroxylase, he/she is unable to metabolize the extra phenylalanine which builds up in the body tissues. The dietary treatment for PKU consists of providing a nutritionally balanced diet containing enough phenylalanine to meet the



needs of a growing child without exceeding the child's limited capacity to utilize phenylalanine. Foods that are high in protein and therefore, high in phenylalanine, are usually excluded from the diet. This includes milk, meat, eggs, cheese and other protein foods. The protein requirement is provided by phenylalanine-free protein substitute. These special dietary products are nutritionally designed to meet the needs for protein, vitamins and minerals. A number of commercial products are available and include: Phenex 1 and

Analog XP (for infants), Phenyl-Free, Phenex 2, Maxamaid XP, Maxamum XP, Periflex, Phlexy-10 and PKU2 (for older patients).

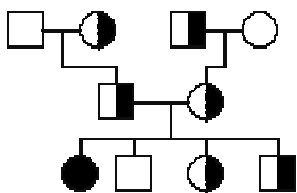
Natural foods provide essential phenylalanine, as well as calories, minerals and vitamins. Fruits and vegetables are essential foods in the diet. Grains and potatoes may be used, but in limited amounts because of their relatively high phenylalanine content.

Sufficient calories must be offered to prevent catabolism. Foods low in protein but high in carbohydrates and fat provide extra calories. Foods containing no protein are considered “free” foods, such as Koolaid, carbonated beverages (NOT DIET), popsicles, lollipops, corn starch, oil and lard. Commercially available low-protein products are an essential part of the diet. Low-protein breads, pastas and baking mixes help make the diet more palatable and interesting. Low-protein recipes have been compiled into cookbooks and reference materials are available that list the phenylalanine content of foods. These resources are important in managing a low-phenylalanine diet. Low-calorie diet foods and medications containing Aspartame are contraindicated because they contain a dipeptide that is metabolized to aspartic acid and phenylalanine.

Growth status, serum phenylalanine and tyrosine levels and nutrient intake must be monitored frequently. Since babies grow so rapidly during the first year of life, serum phenylalanine levels must be obtained twice a week. Parents are trained to obtain blood specimens and keep diet records. Dietary adjustments are made according to the results of the blood specimens. Blood phenylalanine levels should be maintained between 2-5 mg/dl (120-300 $\mu\text{mol/L}$).

Information supporting the need for lifelong treatment of PKU has grown over the past several years. It is the common experience in the metabolic clinics that it becomes increasingly difficult to control phenylalanine levels in older children and adolescents. However, with evidence supporting the need to maintain serum phenylalanine levels below 5 mg/dl (300 $\mu\text{mol/L}$), efforts should be made to continue treatment as long as possible.

Young women with PKU require counseling regarding the strict dietary treatment necessary before conception and throughout gestation. Medical personnel should understand the importance of establishing dietary control before conception, as well as, the scale of intervention required for a successful pregnancy outcome.

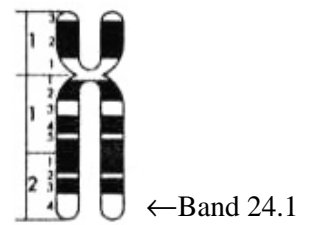


Hyperphenylalaninemia - Genetic and Reproductive Issues

By Bonnie Jeanne Baty, MS, CGC
Genetic Counselor

Hyperphenylalaninemia is a general term to describe too much **phenylalanine** in the body. A deficiency of the enzyme **phenylalanine hydroxylase (PAH)** is often the cause. **Enzymes** are substances in the body that enable chemical reactions to occur. PAH normally acts to convert phenylalanine to tyrosine (two amino acids that are building blocks of protein). A deficiency of PAH causes phenylalanine to build up in the bloodstream, which can harm the body, especially the brain. We use the term **phenylketonuria (PKU)** when the person requires dietary treatment to keep their phenylalanine low enough to avoid brain damage. We use the term **benign hyperphenylalaninemia** when the level without treatment is low enough to avoid brain damage.

The **gene** for *PAH* is on chromosome 12. **Mutations** (changes) in this gene cause the enzyme to work poorly or not at all. PKU can have complete deficiency of the enzyme or a milder deficiency caused by milder mutations in the *PAH* gene. There are over 400 disease-causing mutations in the *PAH* gene.



Everyone has two copies of the *PAH* gene, one inherited from each parent. Most people have two normal copies of the *PAH* gene. About 1 in 60 people in Utah have one normal copy and one copy with a mutation. Such individuals have enough normal PAH to convert phenylalanine and do not have PKU. They are **carriers** for PKU and can pass on their *PAH* mutation to their children. People with PKU have two gene mutations, one inherited from each parent, and have little or no normal PAH. When a couple has a child with PKU, they are both PKU carriers and are at 1 in 4 (25%) risk with each pregnancy to have another child with PKU. Males and females have an equal chance to have PKU. It is important to make sure brothers and sisters born after a child with PKU are adequately screened for PKU (by doing an amino acid blood test). Brothers and sisters of a person with PKU have a 2/3 chance of being a carrier. Carriers can only have a child with PKU if their partner is also a carrier. Other relatives also have an increased risk of carrying a PKU mutation. When a child with PKU grows up, their chance of having a child with PKU is 1 in 120. If their spouse also has a PKU mutation, their risk of having a child with PKU is 1 in 2. For comparison, in the general population a couple's risk to have a child with PKU is 1 in 15,000.

There are two types of carrier tests available. If the person is related to someone with PKU, first the person with PKU is tested to see which genetic mutations they have. If you can identify both of their mutations, then you can test any relative to see if they also inherited one of the PKU mutations. To test an unrelated person such as a spouse, we usually do an amino acid test measuring both phenylalanine and tyrosine.

Because PKU is a treatable disorder, many couples choose not to limit their family size. For parents who are concerned about the risk for PKU in future children, there are several options available to increase the odds of an unaffected child. These options include artificial insemination with a sperm donor screened for PKU, adoption, prenatal diagnosis (amniocentesis or chorionic villus sampling), prevention of pregnancy by birth control or a permanent sterilization procedure, and preimplantation genetic diagnosis (testing the early embryo before implantation and implanting only embryos that will not develop PKU).

Individuals with questions or family members who want carrier testing can call a genetic counselor at 581-8943.